

HELP YOUR FELLOW SCIENTISTS

Grow the Simple Western antibody database by submitting new antibody validation data. Approved submissions receive:

- Free Separation Module (8x25 capillary cartridges) for an antibody against a new target not currently in the Simple Western database.
- Free Detection Module for a new antibody against an existing target or for an existing antibody using a different Separation Mode (Size or Charge).

Visit Database | bio-techne.com/resources/simple-western-antibody-database

Submit to Database | <https://bit.ly/ab-validation-submit>

HOW TO SUBMIT ANTIBODY DATA

1. Set up your plate (see backside for recommendations).
2. Run your antibody validation assay.
3. Annotate the data (see details on back).
4. Submit your antibody validation data with annotated .cbz file on our [online form](#).
5. A notification will be sent once your submission is reviewed.
6. Receive your free separation or detection module.

Refer to checklist for submission requirements



ANTIBODY SUBMISSION CHECKLIST

ANTIBODY (SELECT ONE):

- New antibody for a target not currently in the Simple Western antibody database
- New antibody for an existing target in the Simple Western antibody database
- Existing antibody using a different separation mode (size/charge)

ANTIBODY VALIDATION DATA QUALITY SPECIFICATIONS:

At least one antibody concentration must meet the following data quality specifications

- Signal-To-Noise Ratio (S/N) greater than 20
- Signal height to background level ratio: greater than 3
- Background using HDR less than 1000
- Peak of interest about 80% of total area under the curve (minimal cross reactivity). If protein has multiple peaks, the summed peak areas must be >80%.
- Signal peak height less than 300,000

ASSAY REQUIREMENTS:

- Single target per detection channel (RePlex data and Jess with single targets in different detection channels are accepted)
- 3 antibody concentrations across a reasonably broad concentration range
 - One antibody concentration must meet antibody validation data quality specifications
- 2 Negative Controls
 - No lysate control (i.e. 0.1x sample buffer + 1x fluorescent master mix)
 - No primary antibody control (i.e. sample at highest concentration tested + no primary AB + secondary AB only)

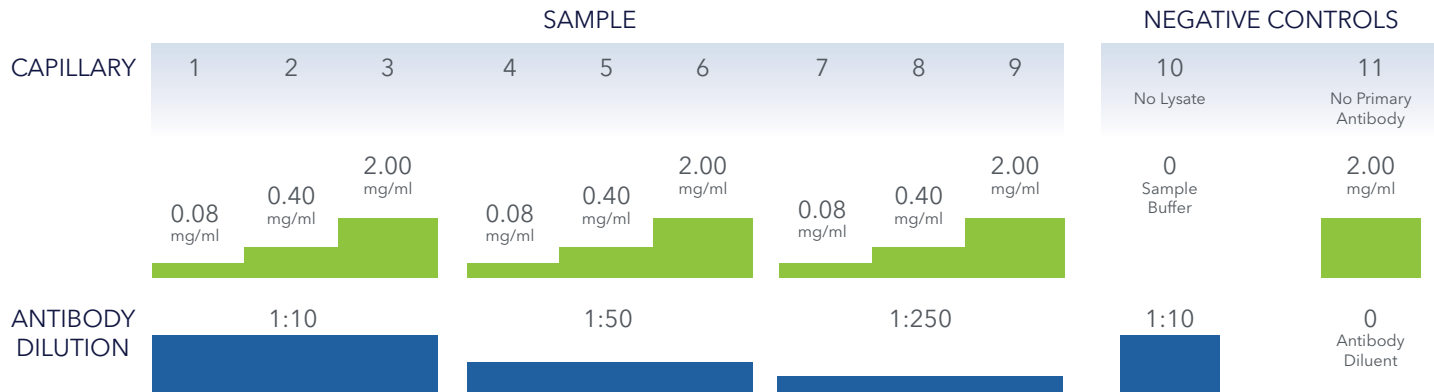
Other negative controls will be accepted at the discretion of ProteinSimple

SUBMISSION FILE FORMAT:

- One data file (.cbz) fully annotated (see back for details)
 - Labeled lane and electropherogram figures with target names and concentrations/dilutions
 - Labeled assay tab with sample and antibody names and concentrations

SIMPLE WESTERN ANTIBODY VALIDATION ASSAY SET UP RECOMMENDATION

This recommendation is for higher quality antibody validation and will take a total of 11 capillaries out of 24. Three antibody concentrations tested against three sample concentrations will utilize nine capillaries (3x3). Two negative controls will utilize two capillaries.



Concentrations listed in the figure above are examples used as a starting point for unknown samples. Exact concentrations should be tailored to each model.

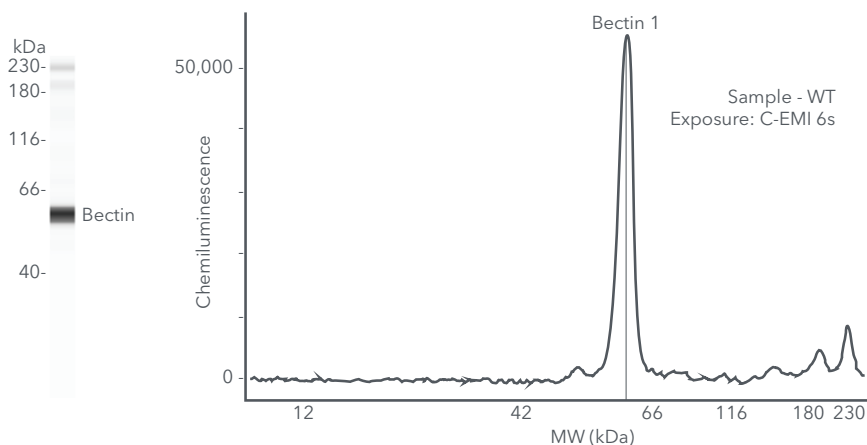
HOW TO ANNOTATE YOUR DATA

Label with the following:

- Target protein band names

Label the Assay tab with the following:

- Sample name
- Sample concentrations
- Primary & secondary antibody names
- Antibody concentration/dilution
- Antibody diluent name



1	2	3	4	5	6	7	8	9	10	11	12
SAMPLE NAME											
Biotinylated Ladder	0.08 mg/ml	0.40 mg/ml	2.00 mg/ml	0.08 mg/ml	0.04 mg/ml	2.00 mg/ml	0.08 mg/ml	0.04 mg/ml	2.00 mg/ml	Sample Buffer	2.00 mg/ml
ANTIBODY DILUENT											
PRIMARY ANTIBODY NAME											
Blocking	1:10	1:10	1:10	1:50	1:50	1:50	1:250	1:250	1:250	1:10	Antibody Diluent
SECONDARY ANTIBODY NAME											
LUMINOL/PEROXIDE											

Learn more about this offer | [biotechne.com/promotions/simple-western-antibody-validation-submission.html](https://www.biotechne.com/promotions/simple-western-antibody-validation-submission.html)